5,273,632, issued December 28, 1993 to Stockham et al. ("Stockham").

Formal Matters

Applicants appreciate the Examiner's time in discussing the subject application in a telephonic interview on May 20, 1996. In the interview, the Examiner stated that the phrase "relative [sic] small" in claim 78 may be indefinite as it is unclear how relatively small is determined. Applicants changed the claim to delete this phrase so the claim recites that the probe intensity will be set to a positive number if the probe intensity is less than or equal to zero. As discussed in the interview, for a number of different reasons, adjusted probe intensities may become negative or zero. Thus, these probe intensities may be set to a positive number (preferably small) to prevent utilizing negative numbers or zero in future calculations (see page 15, lines 23-29). Applicants similarly changed claims 85, 96 and 103 so Applicants believe that these claims are patentably definite.

The Examiner also requested that Applicants discuss U.S. Patent No. 4,965,725, issued October 23, 1990 to Rutenberg et al. ("Rutenberg") and U.S. Patent No. 4,741,043, issued April 26, 1988 to Bacus. Applicants will discuss these references at the end of this Amendment.

In the Office Action, the Examiner rejected claims 1, 3-20 and 45-59 under § 112, second paragraph, and § 103. In order to expedite prosecution, Applicants canceled these claims rendering the rejections moot. Applicants added new claims and the following paragraphs will show how these claims are allowable over the rejections.

Applicants appreciate the Examiner's careful attention to the pending claims. Although claims 1, 3-20 and 45-59 were canceled, the following will briefly describe how the new claims are patentably definite over the § 112 rejections cited in the Office Action. For the Examiner's convenience, Applicants will label the paragraphs according to the labels in the Office Action.

a) In regard to claim 1, the Examiner stated that it is not clear how a probe intensity is associated with a nucleic acid probe. As the Examiner suggested, Applicants amended claim 60 to recite "each probe intensity indicat[es] an extent of hybridization of a nucleic acid probe with at least one nucleic acid sequence including said sample sequence." Accordingly, the rejection does not apply to the new claims.

b,c) Also in regard to claim 1, the Examiner stated that "substantially" and "associated" were indefinite or lack antecedent basis. As claim 60 does not contain these words, the rejection does not apply to the new claims.

d) In regard to claim 1, the Examiner stated that it is unclear how "calling" is defined. Claim 60 recites instead "identifying said unknown base" as was suggested by the Examiner in paragraph e). The Examiner also stated that there seems to a step missing. Applicants do not believe that any steps are missing in claim 60. Accordingly, the rejection does not apply to the new claims.

e) In regard to claim 4, the Examiner stated that the phrase "calling said unknown base as being a base" is unclear. Claim 60 recites instead "identifying said unknown base" as suggested by the Examiner. Additionally, the Examiner stated that it is unclear what a "predetermined ratio value" is. A predetermined ratio value is typically a constant number like 1.2 (see, e.g., claim 63). In the interview, it is believed that the Examiner tentatively agreed that this phrase is patentably definite.

f) In regard to claim 6, the Examiner stated that the "step of sorting" is unclear. Claim 64 recites that a step of sorting probe intensities is done "before said comparing step" (see, e.g., page 14, lines 17-22). Accordingly, the rejection does not apply to the new claims.

g) In regard to claim 9, the Examiner stated that it is unclear how "wild-type" is defined with respect to the "reference sequence." Claim 67 recites that the wild-type probe intensity indicates the extent of hybridization of a complementary probe with the reference sequence. Since the reference sequence is a

known sequence, the wild-type probe is also known (see, e.g., page 20, lines 1-7). Also, the Examiner stated that "each probe intensity of a probe" is unclear. Claim 67 recites instead "each probe intensity of probes" (emphasis supplied). Accordingly, the rejection does not apply to the new claims.

h) In regard to claims 9 and 10, the Examiner stated that the phrases "first ratios" and "second ratios" are not clearly defined. The Examiner suggested that the problem is similar to that of claim 9. As claim 68 recites that "each probe intensity of probes hybridizing with said sample sequence" (emphasis supplied), the rejection does not apply to the new claims.

i) In regard to claim 12, the Examiner stated that the phrase "comparing said ratio of neighboring nucleic acid probes" is unclear. Claim 72 recites instead "comparing said ratio to an analogous ratio of neighboring nucleic acid probes" (emphasis supplied). In the interview, the Examiner stated that she understood what Applicants are claiming and would consider if there is a clearer way to recite this in the claims. Applicants invite the Examiner to contact the undersigned if it would aid in prosecution of the subject application.

j) In regard to claims 13 and 14, the Examiner stated that it is not clear how "a" probe generates more than one intensity. Claims 73 and 74 instead contain the plural "probes." Additionally, the Examiner queried how probe intensities may be compared to statistics. One method described in the specification is to compare the probe intensities to a mean and standard deviation (see also claim 75). As to what the result of the comparison will be, this may depend on the implementation of the invention and the data. Accordingly, the rejection does not apply to the new claims.

k) In regard to claim 16, the Examiner stated that it is not clear what is meant by "related probe intensities." Claim 76 recites that "related probe intensities are from probes that differ by a single base" (see also page 31, lines 14-38). Accordingly, the rejection does not apply to the new claims.

l) In regard to claim 17, the Examiner stated that it is unclear how a background intensity is determined. Applicants respectfully point out that it is not necessary that a claim specifically recite "how" each step may be performed. In general, this is the purpose of the specification. Nevertheless, the Examiner stated that the background intensity may be measured before hybridization of the probes. Additionally, the background intensity may be measured from "blank" probes (see, e.g., page 8, lines 27-31). Accordingly, the rejection does not apply to the new claims.

m-t) same as above

The above has shown that the § 112, second paragraph, rejections in the Office Action do not apply to the pending claims. Therefore, Applicants believe that the claims are patentably definite under § 112.

The Invention

The present invention provides innovative computer-aided methods for identifying unknown bases in nucleic acids. The methods compare probe intensities that indicate the extent of hybridization of a nucleic acid probe with a sample nucleic acid, where each of the nucleic acid probes differ from each other by a single base. After comparing the probe intensities, an unknown base is identified (typically as A, C, G, or T) according to the results of the comparison. In one embodiment, a ratio is calculated between the highest probe intensity and the next highest probe intensity. If the ratio is greater than a predetermined ratio value (e.g., 1.2), the unknown base is identified according to nucleic acid probe that produced the highest probe intensity.

The Cited Art Distinguished

Claims 1, 3-20 and 45-59 were rejected under 35 U.S.C. § 103 as being unpatentable over Fodor in view of Weiss and Stockham. Fodor describes, among other things, pioneering techniques for sequencing by hybridization. However, the Examiner cited Weiss and Stockham for disclosing the base calling

(identifying) methods of the present invention. For the following reasons, these references do not disclose or suggest the present invention as claimed.

Weiss and Stockham are related to nucleic acid sequencing which utilizes nucleic acid ladders which may be formed by well known techniques such as the Sanger dideoxy method or the Maxam and Gilbert method. More specifically, Weiss describes utilizing an enzyme on identical probes that hybridize with tags in the fragments of the nucleic acid ladder. The enzymes convert a fluorogenic substrate (e.g., BBTP) into a fluorescent product in order to enhance the pattern of hybridization (see, e.g., Fig. 1C).

Stockham, more specifically, describes methods of sharpening signal peaks from electrophoretic migration patterns of nucleic acid ladders. Each fragment of the nucleic acid ladder is labeled with a radioactive label which is utilized to identify the position of the fragment on the gel following electrophoresis. As analyzing the migration patterns is time consuming and often error prone, Stockham describes equations and formulas for increasing the accuracy of this process (e.g., sharpening signal peaks).

Weiss and Stockham do not disclose or suggest inputting probe intensities to identify an unknown base where the probe intensities indicate the extent of hybridization of probes differing by a single base and the sample nucleic acid sequence. Claim 60 recites the following:

> inputting a plurality of probe intensities for a plurality of nucleic acid probes, each probe intensity indicating an extent of hybridization of a nucleic acid probe with at least one nucleic acid sequence including said sample sequence, and each nucleic acid probe differing from each other by a single base;

(emphasis supplied). Neither Weiss nor Stockham discloses these limitations.

Initially, Weiss uses a single probe which will hybridize to a tag on the nucleic acid ladder fragments. As such, all of the "probes" in Weiss are identical. Furthermore, the probes in Weiss do not indicate the extent of hybridization but instead are utilized to generate a fluorescent signal which

indicates the location of a fragment on the substrate. Accordingly, it is the location of the fragments that is utilized to sequence a nucleic acid.

Stockham does not utilize probes at all. Instead, Stockham recites that the fragments of the nucleic acid ladder are radioactively labeled. The radioactive signal resulting indicates the position of the fragments on the gel in a way which is similar to Weiss. Accordingly, Stockham also utilizes the location of the fragments to sequence a nucleic acid.

In stark contrast, the present invention compares probe intensities that indicate the extent of hybridization of probes differing by a single base and the sample nucleic acid sequence. Claim 60 recites the following:

> said computer system comparing said plurality of probe intensities; and
> identifying said unknown base according to results of said comparing step.

In the Office Action, the Examiner stated that it would have been prima facie obvious to one of ordinary skill in the art to use the computer algorithms of Weiss and Stockham to interpret that data from the sequencing by hybridization described by Fodor. More specifically, the Examiner stated that one could "call" a site based on the intensity of a signal produced by a probe at that site and thus assign an identity to that site. Applicants disagree.

Weiss and Stockham relate to vastly different technologies than the pioneering advances of Fodor. Weiss and Stockham are directed to identifying the location of a fragment of a nucleic acid ladder. In the present invention, the locations of the hybridized probes are known and, as such, the computer algorithms of Weiss and Stockham would indeed seem to teach away from the present invention which is directed to calling an unknown base according to probe intensities from nucleic acid probes that differ by a single base.

As Weiss and Stockham do not disclose or suggest all the limitations of claim 60, the claim is patentably distinct over the references. All the other pending claims contain similar limitations. Therefore, Applicants request that all the

pending claims be passed to issue.

Other Claims

Independent claims 81, 88 and 99 recite specific methods of identifying unknown bases. Details on specific embodiments of these methods may be found in the specification under the headings "Intensity Ratio Method," "Reference Method" and "Statistical Method." These claims recite methods that are patentable for at least the same reasons as above. Additionally, these claims include further limitations that make them further patentably distinct.

Claim 81 recites that a ratio of a higher probe intensity and a lower probe intensity is calculated. Then, the unknown base is identified according to the probe that had the higher probe intensity if the ratio is greater than a predetermined ratio value. Weiss and Stockham simply do not disclose or suggest this method. Accordingly, claims 81-87 are patentably distinct.

Claim 88 recites that probe intensities from a first set of probe intensities from probes hybridizing with a reference nucleic acid sequence and a second set of probe intensities from probes hybridizing with a sample nucleic acid sequence are compared. Based on this comparison, the unknown base is identified. Weiss and Stockham do not disclose or suggest this method. Accordingly, claims 88-98 are patentably distinct.

Claim 99 recites that a probe intensity of a nucleic acid probe hybridizing with a sample sequence is compared to statistics from nucleic acid probes hybridizing with a reference sequence. Based on this comparison, the unknown base is identified. Weiss and Stockham do not disclose or suggest this method. Accordingly, claims 99-105 are patentably distinct.

Additionally Cited Art

In the Office Action, the Examiner cited Rutenberg and Bacus as relevant to programs designed to distinguish ratios of intensities of light. Although in the interview the Examiner stated that these references may be nonanalogous art, she

requested that these references be discussed by Applicants. The following will show that these references do not teach or suggest the present invention regardless of whether the references are analogous art.

Rutenberg describes a two stage neural network system for classifying cells on a slide, e.g., for detecting cervical cancer. In a first stage, the neural network classifies cells or objects which are pre-malignant and malignant. However, the first stage may include other nonmalignant objects like cell clumps, debris, leucocytes, and mucus. A second stage of the neural network is utilized to distinguish the pre-malignant and malignant cells from the nonmalignant objects. As Rutenberg describes methods of distinguishing objects on a slide utilizing neural networks, the reference does not disclose or suggest the base calling methods of the present invention.

Bacus describes a method for overcoming staining variations among cells for analysis, e.g., for cancer diagnosis and prognosis. Conventional staining mechanisms may have variations among experiments so reference cells are placed on the slides with the specimen cells. After staining, the imaging apparatus is calibrated according to the reference cells. The specimen cells are then analyzed to determine characteristics such as nuclear optical density. As Bacus describes methods of calibrating imaging apparatus for analyzing cells on a slide, the reference does not disclose or suggest the base calling methods of the present invention.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (415) 326-2400.

Respectfully submitted,

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